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ANALYSIS OF DIATOM COMMUNITIES IN AN ACID-MINE-DRAINAGE-IMPACTED SUBWATERSHED IN SOUTHEASTERN OHIO

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ABSTRACT

Acid mine drainage (AMD) impacts numerous streams worldwide. During June of 2000, 18 stream segments located within the Black Fork subwatershed in southeastern Ohio were sampled for diatom flora and critical environmental parameters. This area has a history of prolific coal mining and many of the region's lotic systems are inundated with AMD. In this region, many of the abandoned mines have been reclaimed using various techniques. The goal of this study was to determine if diatom assemblages could provide evidence of the progress and effectiveness of reclamation activities with respect to biotic integrity of aquatic systems. Through the cross-referencing of various exploratory techniques three distinct groupings of sites were depicted, each containing similar relative abundances of important diatom taxa. Group I sites were heavily impacted by AMD and were dominated by *Eunotia exigua* (Brébisson *ex* Kützing) Rabenhorst. Group II sites were moderately impacted by AMD and had diatom assemblages of *Achnanthidium minutissimum* (Kützing) Czarnecki, and *Brachysira vitrea* (Grunow) R. Ross *in* B. Harley. Group III sites contained relatively unimpacted headwater regions in the subwatershed with diatom assemblages dominated by *A. minutissimum*. The diatom assemblages were useful in identifying certain sites that prior to this study were thought to be major contributors of AMD, but yielded taxa characteristic of intermediate conditions, suggesting that these sites fluctuate in water chemistry throughout the year. The unique diatom assemblages in these intermediate, oscillating streams (Group II) pinpointed cryptic pollution sites with a greater degree of accuracy than environmental parameters alone.

Introduction

Coal mining has been a vital industry to the Appalachian region since the early 1800s. The coal-mining process can lead to a variety of environmental impacts including hydrogeochemical effects, subsidence, and mine gas emission (Banks et al., 1997). While all of these examples represent serious dilemmas, the predominant environmental problem associated with coal mining is acid mine drainage (AMD). AMD is defined as runoff of a dilute solution of sulfuric acid and iron sulfate in the ferrous and ferric form (USEPA, 1983). This drainage results from the exposure of mine subsidence and spoils, which contain substantial quantities of pyritic minerals (FeS_2), to oxidation (both abiotic and biotic). AMD can have a negative effect on the diversity of aquatic ecosystems due to toxicity from increased hydrogen ion activity, dissolved metals, and an influx of metal precipitates which degrade available habitat (Herricks, 1977; Leatherman and Mitsch, 1978; Mulholland et al., 1986; Wieder et al., 1990; Skousen et al., 1994; Keating et al., 1996; Planas, 1996; Verb and Vis, 2000, 2005). There are an estimated 16,090 km of AMD-impacted stream systems in the Appalachian region (Dugan, 1975; Starnes, 1985).

Despite reductions in species richness in AMD-impacted systems, a variety of organisms can be employed as biological indicators in such investigations. Of these organisms, the algae, particularly diatom assemblages, have several advantages over other organisms as biomonitors because they are abundant and have cosmopolitan distributions within a watershed (i.e., Lowe and Pan, 1996; Stevenson and Pan, 1999). Furthermore, the diatom communities are excellent biological indicators of surface water acidity and have shown promise in the evaluation of AMD remediation (Battarbee et al., 1999; Verb and Vis, 2000, 2005).

The goals of this investigation were to: 1) determine the influence of AMD on the benthic diatom assemblages within a small subwatershed and, 2) explore the amount of resolution the diatom assemblages provide when assessing the remediation success of abandoned coal mines within a small subwatershed.

Methods

The sedimentary rocks of the Moxahala watershed were laid down from the Mississippian to the Permian periods and consist primarily of sandstone, shale, limestone, and Pennsylvanian-age coal. There are also glacial-outwash deposits of clay, silt, and gravel of Quaternary age. The Moxahala drainage basin has an average pH of approximately 6.0 in its headwater regions, although near its confluence with the Muskingum River the pH decreases to 4.5 (Eberhart, 1998; Kocsis, 2000). The Moxahala watershed suffers from a high degree of both chemical and sedimentation pollution and is currently one of the most highly AMD-impacted hydrologic units in the state of Ohio (USDA, 1985; Eberhart, 1998). These studies indicated that there were contaminations of AMD in approximately 60 percent of the watershed's 267 km of tributaries and 132 km of excessive sedimentation. Coal was mined in approximately 68 km^2 of the watershed (USDA, 1985). The Black Fork subwatershed represents one third of the total Moxahala watershed ($\sim$78 km^2) and has three main contributors: Dry Run, Ogg Creek, and Bennett Run. Black Fork provides approximately 25 percent of the total flow to Moxahala Creek. However, it is the least contaminated of four major tributaries discharging into Moxahala Creek, contributing between 15 and 20 percent of the total iron and sulfate loading (Eberhart, 1998; Kocsis, 2000). The Ohio Department of Natural Resources-Division of Mines and Reclamation (ODNR-DMR) determined that the Black Fork subwatershed would be the focus of various reclamation efforts (e.g., wetlands) and a detailed watershed analysis because it has the greatest potential for full recovery (Kocsis, 2000).

The Muskingum Mining Company mined 10 km^2 of No. 6 coal prior to 1956 from the Misco Mine. The company deposited two large refuse piles (Misco gob piles) on both banks of Bennett Run. Since the Misco gob piles represented the primary sources of AMD in the Black Fork subwatershed they were partially reclaimed in 1990. The reclamation efforts included excavation and regrading of one gob pile along with the stifling of 16,820 m^3 of burning coal refuse before the pile was capped. Topsoil was replaced and the site was revegetated. The second burning (9,175 m^3) refuse pile was left unreclaimed due to a lack of funds (Kocsis, 2000).

The second major AMD source in the Black Fork subwatershed arises from an underground mine seep near Tropic, Ohio. In 1994 the ODNR-DMR designed and constructed a treatment wetland (Tropic Wetland). The AMD seep is funneled into an anoxic limestone drain (ALD) and then travels through a pipe where it discharges into a sedimentation pond. Subsequently, the water is directed into one of twelve wetland cells. Each cell has a base of peat mixed with lime in order to provide suitable substrate for bacterial colonization and increased alkalinity. Corrugated fiberglass sheets separate each cell and the cells empty into collection channels, which then discharge into one of two outlets to Black Fork (Kocsis, 2000).

Physical and chemical analysis

Eighteen preselected sites were visited in June 2000. These sites represented permanent sampling stations for the Black Fork subwatershed investigation initiated by ODNR-DMR (Table 1 and Figure 1).

At each sampling site, a 20-m stream segment was measured for maximum wetted width at three randomly determined locations. The average thalweg depth (deepest part of channel) was calculated every 2 m along the 20-m transect (11 total measurements). Specific conductance, pH, and temperature were measured at each site using handheld probes. Stream water was collected at each site in 1000 ml, 500 ml, and 250 ml containers for water chemistry and placed on ice for transportation. The 1000-ml and 500-ml samples were then shipped to the ODNR-DMR analytical laboratory in Cambridge, Ohio for analysis of pH, total acidity, total alkalinity, total suspended solids, sulfate, chloride, total calcium, total magnesium, total sodium, total potassium, total iron, total manganese, total aluminum, and hardness. Within eight hours of collection, stream water from the 250 ml container was analyzed for turbidity (using a Hach 2100P™ turbidity meter), and water samples were filtered using Whatman GF/F µm filters for nitrate (NO_3-N), soluble reactive phosphorus (SRP), and sulfate (SO_4^{2-}), and 0.45 µm filters for silica (SiO_2). These analyses were conducted with a Hach DR/890™ colorimeter with standard protocols and powder pills from Hach Company (Anonymous, 1997).

Diatom sampling and analysis

At each site, five rocks were randomly selected from a transect placed across a riffle area and a 5.0 cm^2 area on each rock was scraped for periphyton by securing a rigid rubber O-ring, scouring with a stiff toothbrush, and rinsing with stream water. Material from the five scrapes was combined and a composite sample of

Table 1. Descriptions of 18 sampling locations from within the Black Fork subwatershed as defined by Kocsis (2000). AMD = acid mine drainage, ALD = anoxic limestone drain.

Station abbreviation	Description
BF1	Black Fork before entering Moxahala Creek
BF10	Whitehouse Seep before entering Black Fork
BF13	Black Fork before AMD contamination
DR1	Dry Run before entering Black Fork
DR2	Seep No. 2 before entering Dry Run
DR3	Seep No. 1 before entering Dry Run
DR7	Dry Run before merging with seeps
OC1	Ogg Creek before entering Black Fork
OC4	Ogg Creek before AMD contamination
BR1	Bennett Run before entering Ogg Creek
BR3	Discharge from burning gob pile into Bennett Run
BR9	Bennett Run before burning gob pile
BR14	Bennett Run before AMD contamination
TW0	Tropic Wetland well—before ALD
TW1	Outlet from ALD to Tropic Wetland sedimentation pool
TW2	Channel leading from sedimentation pool to Tropic Wetland
TW3	Tropic Wetland—discharge to Black Fork
TW4	Tropic Wetland—discharge to Black Fork

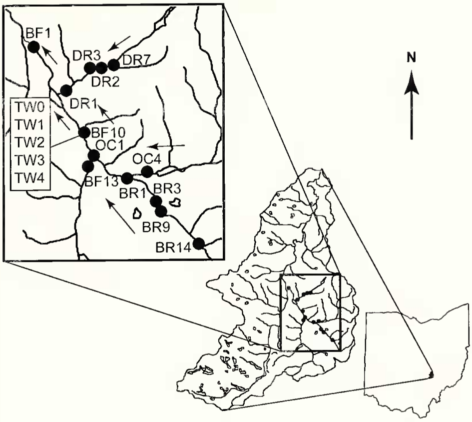

Figure 1. Map indicating the location of the Moxahala watershed in southeastern Ohio, the Black Fork subwatershed within the Moxahala watershed, and the sampling locations for the current investigation. Arrows indicate direction of water flow in the tributaries. The inset box is 5.25-km wide.

20 ml was preserved with 2.5 percent $CaCO_3$-buffered glutaraldehyde for later identification and enumeration of benthic diatoms.

Palmer-Maloney counting chambers were used to determine abundances and basic morphologies of living diatoms (presence/absence of chloroplast) in each sample. Preserved diatom samples were homogenized and a 10 ml subsample was cleaned using 30 percent H_2O_2 and concentrated HNO_3 (Stoermer et al., 1995). The clean diatom sample was suspended in glass-distilled water, placed in evaporation chambers similar to those devised by Battarbee (1973), and prepared on slides using NAPHRAX. For each sampling date, 600–1000 valves were counted along 18-mm transects at 1000× using a BX40 Olympus microscope.

Diatoms were identified to species using Patrick and Reimer (1966, 1975) and Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b). Relative abundance, the Shannon index of diversity, density, and numerical species richness were calculated for each subsample (Magurran, 1988, p. 7–45).

Statistical analyses

Data were initially inspected for adherence to the assumptions of univariate and multivariate normality. Non-normal data were transformed using $\log_{10}$ and $\log_{10} + 1$ to meet normality assumptions. Exploratory analyses were conducted with both species data and environmental data from each site and date to investigate the relationship of the streams based on the degree of AMD contamination. Unweighted pair-group mean average (UPGMA) cluster analyses were conducted using NCSS 2000 (Hintze, 2000) statistical software, environmental data were examined using principal components analysis (PCA) and correspondence analysis (CA) for species data using CANOCO (ter Braak and Šmilauer, 1998).

Detrended correspondence analysis (DCA) was used to determine the variation in the algal data sets. Based on the gradient lengths along the first DCA axis it was determined that canonical correspondence analysis (CCA, unimodal response model) would be the proper ordination technique to employ (ter Braak and Prentice, 1988). The patterns observed from exploratory statistics (UPGMA, PCA, CA) were compared with the results of canonical correspondence analysis (CCA) using CANOCO (ter Braak and Šmilauer, 1998) to determine if there were distinguishable groups of taxa and stream sites that could be used in classification. Through the cross-referencing of these techniques, the stream sites were partitioned into multivariate groups. CCA was conducted using abundant diatom taxa (relative abundance $\geq$ 0.5 percent, present at $\geq$ 2 sites). Environmental data were analyzed and $\log_{10}$-transformations applied to those parameters with skewed distributions. Those variables with high correlation coefficients ($r > 0.85$) and variance inflation factors (> 10) were eliminated (Pan et al., 1996; ter Braak and Šmilauer, 1998). The significance of the first 4 CCA axes was tested using Monte Carlo permutation tests (1000 permutations, $\alpha = 0.05$). To further test the influence and significance of important environmental variables from the initial CCA, a series of constrained CCAs were conducted along with Monte Carlo permutation tests (ter Braak and Šmilauer, 1998).

Two unbalanced multivariate analyses of variance (MANOVA) were conducted using SAS (SAS Institute Inc., 1996). Stream group from multivariate analyses was a fixed effect with measurements of the algal assemblages (species diversity and richness) and selected environmental parameters as response variables. Bonferroni (Dunn) multiple comparison tests were employed to investigate significant differences among the category types.

Results

CCA results

Of the original 22 variables measured from the stream sites, 14 were excluded due to problems associated with autocorrelation and variance inflation. A total of 105 diatom species were identified in the samples recovered from the Black Fork subwatershed. Of these, 62 taxa were included in the multivariate analyses because they had a relative abundance $\geq$ 0.5 percent and were present at $\geq$ 2 sites. Site TW0 (wetland well) contained no diatom taxa and thus was eliminated from the CCA analyses. The first four axes of the periphyton CCA were statistically significant (Monte Carlo permutation, $P = 0.02$) and explained 45.7 percent of the species variance (Table 2). The correlation between the diatoms and physical and chemical variables was highest along the first and second axes ($r = 0.69, -0.88$) and dropped in the following axes ($r = 0.53, -0.66$; Table 2). There was an influence of mine drainage along CCA axes 1, 3, and 4 (Table 2). The first axis was strongly correlated with pH ($r = -0.88$) while the second axis was influenced by temperature ($r = 0.83$; Table 2). The eight variables included in this analysis accounted for 72.7 percent of the total explained diatom species variance (Table 2). Constrained CCAs performed on these eight environmental variables revealed that two of these variables, pH and Fe, were statistically significant ($P < 0.05$; Table 3).

Site distributions and characteristics

Cross-referencing of the various exploratory techniques (i.e., clustering, CA, PCA, CCA) was employed to determine the

Table 2. Summary of canonical-correspondence-analyses (CCA) results for the first four axes of the diatom set. λ = eigenvalue; S = percent variance explained by the corresponding axis; SER = species-environment relation; TVE = total variance explained; r = correlation coefficient between axis and influential environmental parameters. All axes were statistically significant ($P < 0.05$) as determined by Monte Carlo permutation tests (1000 bootstrap replicates).

Axis	λ	S	SER	Environmental parameter (r)
I	0.449	21.3	33.9	pH (−0.88), Fe (0.69)
II	0.193	9.3	14.7	Temperature (0.83)
III	0.156	8.7	13.9	Turbidity (0.60), Fe (0.53), Width (−0.53)
IV	0.127	6.4	10.2	Al (−0.66)
	TVE	45.7	72.7	

Table 3. Constrained CCA results of environmental variables employed in the initial CCA.

Variable	Percent variance explained	Monte Carlo P-value
pH	16.9	.0010
Fe	13.6	.0070
Width	9.5	.0509
Turbidity	8.5	.0899
Temperature	7.8	.1389
PO_4	7.7	.2008
Al	7.1	.2527
Depth	5.1	.7283

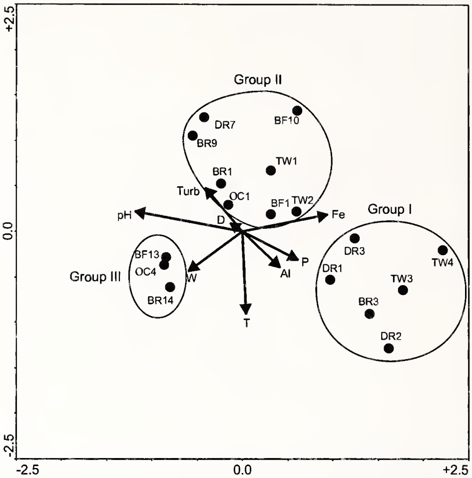

Figure 2. Diatom-based CCA biplots of Black Fork subwatershed stream sites with environmental variables represented by arrows. D = thalweg depth, P = orthophosphate, T = temperature, Turb = turbidity, W = maximum wetted width.

indicated three groups of sites. Due to the repetitive nature of these analyses only the results of the CCA are presented since this analysis type incorporates both the environmental and diatom data sets (Figure 2). In all analyses the composition of the three multivariate groups remained in relative agreement. Stream site variation that occurred was primarily due to the switching of site positions within each multivariate group. Group I was composed of sites heavily impacted by AMD. The stream water at these sites was the lowest in pH and turbidity (Table 4). Heavily impacted AMD sites (Group I) also had the highest concentrations of dissolved metallic ions (Al, Fe, Mn), specific conductance, and SiO_2 (Table 4). The second multivariate grouping was comprised of several remediated and unreclaimed sites, that were moderately impacted by AMD (Figure 2). These sites were moderately acidic with low buffering capacities (Table 4). Group II sites had moderate concentrations of Al, Fe, Mn, specific conductance, and SO_4^{2-} which were significantly higher than the levels found in Group III, but not Group I (Table 4). Group III was an agglomeration of three headwater sites with little to no AMD impact. These sites displayed circumneutral pH levels with low concentrations of AMD-related variables (i.e., Al, Fe, Mn). Group III sites also had the highest levels of total alkalinity recorded in this study (Table 4).

The heavily impacted AMD sites located in Group I were dominated by *Eunotia exigua*, with additional assemblage composition from *Nitzschia capitellata*, *Pinnularia subcapitata*, *Frustulia rhomboides*, and *Eunotia steineckii* (Appendix 1). Moderately impacted AMD sites contained in Group II displayed diatom assemblages containing *Achnanthidium minutissimum*, *Pinnularia obscura*, *Brachysira vitrea*, *Eunotia exigua*, and *Nitzschia inconspicua* (Appendix 1). The relatively unimpacted headwater sites (Group III) had a flora of *A. minutissimum*, *N. acicularis*, *N. dissipata*, *Planothidium lanceolatum*, and *Rhoicosphenia curvata* (Appendix 1).

Multivariate Groups I and II had levels of diatom species richness and diversity that were significantly (MANOVA: $P < 0.05$) lower than the measurements from Group III (Figure 3A, B). In addition, Group I was significantly lower (MANOVA: $P < 0.05$) than Group II in both of these measurements as well.

Discussion

CCA results

There was a clear delineation between the three multivariate groups, with little gradation between them. The influence of pH on these systems was not unexpected, given the wide range of pH values (2.7–7.2) and the importance of this variable in other lotic studies from the region (Pan et al., 1996; Verb and Vis, 2000, 2001, 2005). However, along the second CCA axis the influence of temperature was unanticipated. The lower water temperatures were predominately associated with the headwater systems in Group I, water discharging from the ALD, and many of the systems receiving AMD-contaminated groundwater (e.g., seeps). Despite the perceived importance of temperature along the second CCA axis this variable was not statistically significant when examined with a constrained CCA. The 30.6 percent of the diatom variance explained by the first two CCA axes is higher than in other lotic algal studies (12.0–21.1 percent), but the inflation of this value may be attributed to the small number of samples in this study (17 containing diatoms).

Table 4. Summary of descriptive statistics for selected environmental variables (median value with ranges) and for 17 sample sites with diatoms in the Black Fork subwatershed. Stream categories according to ordinations groups described in the text. NTU = Nephelometric Turbidity Units; SRP = Soluble Reactive Phosphorus; TDS = Total Dissolved Solids; TSS = Total Suspended Solids; * = variables used in multivariate analyses.

Parameter	Group I	Group II	Group III
Al* (mg/l)	16.0 (1.2–212.0)	4.1 (2.3–17.3)	0.65 (0.54–2.88)
Specific conductance ($\mu S \cdot cm^{-1}$)	2735 (1020–3250)	828 (331–2300)	264 (250–298)
Fe* (mg/l)	44.9 (13.7–645.0)	37.9 (1.91–326.0)	0.4 (0.2–0.4)
Maximum wetted width* (m)	6.0 (0.9–7.2)	2.2 (1.1–7.2)	5.7 (5.3–8.0)
Mn (mg/l)	3.8 (1.7–5.3)	1.1 (0.5–4.9)	0.2 (0.1–0.2)
NO_3 (mg/l)	0.5 (0.1–4.6)	0.0 (0.0–0.9)	0.0 (0.0–0.1)
pH*	2.84 (2.68–3.07)	5.54 (5.19–6.42)	7.11 (6.97–7.23)
SRP* (mg/l)	0.49 (0.14–0.75)	0.26 (0.20–0.79)	0.24 (0.20–0.26)
SiO_2 (mg/l)	30.7 (16.3–63.9)	8.6 (2.6–26.9)	5.0 (3.8–5.9)
SO_4^{2-} (mg/l)	1474 (410–3013)	389 (85–1605)	31 (31–35)
TDS (mg/l)	2120 (597–4540)	628 (215–2540)	152 (148–160)
TSS (mg/l)	15 (9–97)	58 (10–184)	9 (5–10)
Temperature* (°C)	20 (16–23)	16 (14–18)	15 (14–17)
Thalweg depth* (cm)	8 (4–86)	10 (4–26)	9 (8–42)
Total acidity (mg/l)	186 (114–511)	52 (0–317)	0 (0)
Total alkalinity (mg/l)	0 (0)	8 (4–57)	85 (72–99)
Turbidity* (NTU)	3.8 (0.8–5.5)	100.4 (7.8–458.0)	11.7 (3.4–30.0)

Multivariate groups

Taxa associated with Group I are very characteristic of acidic conditions. *Eunotia exigua*, *E. steineckii*, *N. capitellata*, *P. subcapitata*, and *F. rhomboides* are all taxa that have been reported from acidic lotic conditions (Bennett, 1969; Hancock, 1973; Patrick, 1977; Kelly, 1988; Planas, 1996; DeNicola, 2000; Verb and Vis, 2000, 2005). While Group II showed moderate effects of AMD (e.g., depressed levels of species richness), sites within this grouping contained rather unique diatom taxa in the assemblages. The importance of *A. minutissimum*, *B. vitrea*, and *P. obscura* at these sites was interesting because these are the dominant taxa in lotic systems in this region which oscillate in water chemistry from acidic to circumneutral (Verb and Vis, 2000, 2005). *Pinnularia obscura* was an especially intriguing taxon given that it is often recorded as a common aerial diatom, typical of somewhat brackish water conditions (Van Dam et al., 1994; Johansen, 1999). However, several investigations report this taxon being found in a wide range of freshwater environments (Patrick and Reimer, 1966; Troeger, 1978, 1983; Pfiester et al., 1979). Further investigation is required to determine if *P. obscura* is indicative of these oscillating water conditions and/or an aberrant aerial taxon being flushed into the system via surface runoff. Group III sites contained diatom taxa, such as *N. acicularis*, *N. dissipata*, *P. lanceolatum*, and *R. curvata*, that were generally intolerant of acidic conditions (Verb and Vis, 2000).

AMD pollution sources

Kocsis (2000) reported that there were four major AMD sources in the Black Fork subwatershed: Tropic Wetland (TW0-TW4), Whitehouse Seep (BF10), Dry Run (DR1, DR2, DR3, DR7), and the Misco burning gob pile (BR3). The Tropic Wetland was considered to be operating sufficiently (Fe removal and neutralization of mineral acidity) with the exception of sulfate removal (Kocsis, 2000), however, there was a definite spatial divergence between the diatom assemblages found near the ALD and where the wetland discharges into Black Fork. The two sites near the ALD discharge (TW1 and TW2) represent moderate AMD impact, but at the end of the wetland treatment (TW3 and TW4), diatom assemblages indicate these sites are heavily

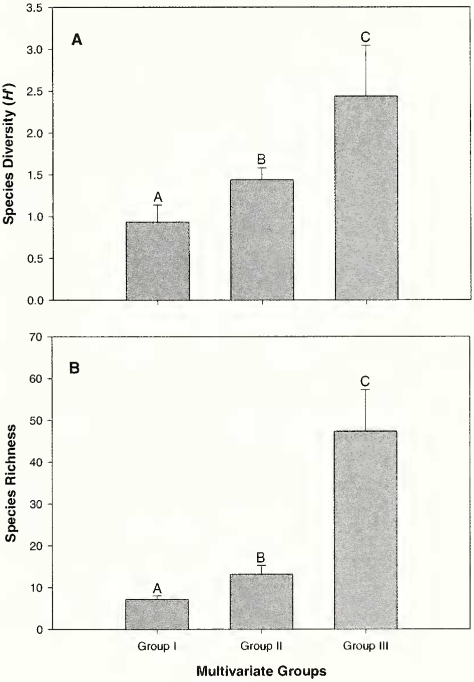

Figure 3. Mean values (+SE) for selected diatom assemblage variables. Bars with the same letters are not significantly different ($P < 0.05$).

impacted by AMD. TW3 and TW4 are dominated by *E. exigua* (73–84 percent relative abundance), a taxon prevalent in heavily impacted AMD sites of this region (Verb and Vis, 2000, 2005). It appears that the ALD is effective at raising the pH of the water discharging from it and the sedimentation pool (TW1 and TW2). It has been postulated that as this treated water moves through the wetland hydrolysis, precipitation of the oxidized Fe generates additional hydrogen ions (Kocsis, 2000), resulting in the acidic conditions and flora found at the wetland discharge points (TW3 and TW4). Furthermore, it has been noted that precipitating Fe floc may alter the physical environment in which the diatoms reside and the same may be true for these sampling sites (Verb and Vis, 2005). However, another plausible explanation for the AMD within water discharging from the Tropic Wetland may be the intrusion of AMD-contaminated groundwater which is either bypassing or not being neutralized by the ALD and microbial processes. Nevertheless, further investigation into the hydrologic regime around and within the Tropic Wetland is needed to confirm or deny these suspicions.

The Whitehouse Seep (BF10) represented the largest flow contributor to Black Fork but was considered third on the list of AMD sources in this basin (Kocsis, 2000). However, the diatom assemblage and environmental parameters depicted this particular site within Group II, which is comprised of moderately impacted AMD sites. The dominance of the diatoms *A. minutissimum*, *B. vitrea*, and *P. obscura*, along with low levels of total alkalinity (10.4 mg/l), are indicative of intermediate AMD disturbance and perhaps oscillating water chemistry (fluctuating between acidic and circumneutral pH) (Verb and Vis, 2000, 2001, 2005).

The Misco burning gob pile (BR3), contributing < 10 percent of the flow to Black Fork, was considered one of the more severe AMD point discharges in the Black Fork subwatershed. Diatom taxa were highly indicative of heavily impacted AMD conditions with a flora dominated by *E. exigua*, *N. capitellata*, and *P. subcapitata*.

The final major source of AMD in the Black Fork subwatershed was Dry Run. In its headwater region (DR7) this tributary is only marginally impacted by AMD as indicated by its location in multivariate Group II. At DR7 the dominant diatom taxa are *A. minutissimum*, *E. exigua*, *B. vitrea*, and *N. inconspicua*, suggesting that at this site (DR7) the system may be oscillating in nature. However, after the two AMD seeps (DR2 and DR3) merge with Dry Run downstream, there was a degradation in water quality and a corresponding reduction in species richness and a rise in the dominance of *E. exigua* (50–95 percent relative abundance) in the diatom assemblages.

In conclusion, diatoms were useful at detecting and characterizing the various AMD intensities at sites throughout the Black Fork subwatershed. Furthermore, some sites that were thought to be major contributors of AMD supported a diatom assemblage that was representative of intermediate AMD (i.e., BF10). Evidence from this study provides further support that diatom assemblages are useful in characterizing AMD pollution at a variety of spatial scales (small subwatershed to ecoregion). In addition, diatoms are a useful tool in pinpointing problematic AMD-pollution sectors and may prove to be useful organisms in tracking the impacts of restoration efforts.

Acknowledgments

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Appendix 1. Mean relative abundance of dominant diatom taxa for each multivariate group of stream sites sampled in the Black Fork subwatershed.

Taxon	Multivariate Group		
	I	II	III
Achnanthes deflexa Reimer	0.00	0.76	0.55
Achnanthidium minutissimum (Kütz) Czarn.	1.77	29.20	44.56
Amphipleura pellucida (Kütz.) Kütz.	0.00	0.16	0.10
Amphora montana Krasske	0.00	0.35	0.10
Brachysira vitrea (Grunow) Ross	0.48	9.10	0.00
Cocconeis pediculus Ehrenb.	0.00	0.16	1.00
C. placentula Ehrenb.	0.00	0.00	0.22
Craticula halophila (Grunow) Mann	0.00	2.29	0.11
Cymbella affinis Kütz.	0.00	1.85	0.52
Denticula kuetzingii Grunow	0.00	0.33	0.09
Diatoma vulgare Bory	0.00	0.16	0.10
Encyonema lange-bertalotii Krammer	0.00	0.00	0.20
E. minuntum (Hilse *ex* Rabenh.) Mann	0.00	0.35	1.01
E. silesiacum (Bleisch *ex* Rabenh.) Mann	0.00	0.33	0.41
Eunotia curvata (Kütz.) Lagerst.	0.77	0.20	0.21
E. exigua (Bréb.) Rabenh.	71.50	5.75	0.63
E. steineckii Petersen	1.57	0.21	0.00
Fragilaria capucina Desm.	0.21	1.23	1.45
F. tenera (Smith) Lange-Bert.	0.00	0.00	0.43
Frustulia rhomboides (Ehrenb.) DeToni	5.11	0.00	0.00
Gomphoneis olivacea (Hornemann) Dawson	0.00	0.33	1.53
Gomphonema sp.	0.00	0.66	0.50
G. minuta Agardh	0.00	0.00	0.71
G. parvulum (Kütz.) Kütz.	0.24	0.45	0.41
Luticola mutica (Kütz.) Mann	0.24	0.35	0.00
Melosira varians Agardh	0.00	2.08	2.48
Meridion circulare (Grev.) Agardh	0.00	0.00	0.95
Navicula cincta (Ehrenb.) Ralfs	0.00	0.20	1.15
N. clementis Grunow	0.00	0.68	0.00
N. cryptocephala Kütz.	0.00	0.51	0.53
N. cryptonella Lange-Bert.	0.95	0.00	1.40
N. goeppertiana (Bleisch) H. L. Smith	0.06	0.78	0.00
N. gregaria (Donkin)	0.00	0.00	2.28
N. lanceolata (Agardh) Kütz.	0.00	0.82	2.43
N. minuscula Grunow	0.00	0.35	0.51
N. viridula (Kutz.) Ehrenb.	0.00	0.00	0.30
Nitzschia acicularis (Kutz.) W. Sm.	0.00	0.00	4.25
N. amphibia Grunow	0.00	3.11	0.92
N. capitellata Hust.	8.46	3.17	0.31
N. clausii Grunow	0.00	1.07	0.10
N. constricta (Kütz.) Grunow	0.00	0.00	0.52
N. dissipata (Kütz.) Grunow	0.00	0.66	3.35
N. fonticola Grunow	0.00	0.00	0.38
N. inconspicua Grunow	0.00	3.96	1.49
N. linearis (Agardh) W. Sm.	0.00	0.79	1.96
N. microcephala Grunow	0.00	0.00	0.20
N. nana Grunow	0.00	0.82	0.18
N. palea (Kütz.) W. Sm.	0.00	1.33	1.81
N. perminuta (Grunow) M. Perag.	0.00	2.42	1.70
N. recta Hantzsch	0.00	0.35	0.41
Pinnularia microstauron (Ehrenb.) W. Sm.	0.06	0.13	0.00
P. obscura Krasske	1.74	14.79	0.00
P. subcapitata W. Greg.	5.93	1.77	0.00
Planothidium lanceolatum (Bréb.) Round & Bukht.	0.00	0.84	2.84
Reimeria sinuata (Greg.) Kociolek & Stoermer	0.00	0.33	1.29
Rhoicosphenia curvata (Kütz.) Grunow	0.00	0.33	0.33
Sellaphora pupula (Kütz.) Mereschk.	0.00	0.21	0.10
Surirella angusta Kütz.	0.00	0.00	0.20
S. brebissonii Krammer & Lange-Bert.	0.00	0.49	2.40
S. ovalis Bréb.	0.00	0.20	0.21
Synedra delicatissima W. Sm.	0.00	0.16	0.53
S. ulna (Nitzsch) Ehrenb.	0.45	1.24	1.82